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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/901,419	07/09/2001	Calvin C. Hale	UMO 1531.1	7363

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SENNIGER POWERS LEAVITT AND ROEDEL
ONE METROPOLITAN SQUARE
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ST LOUIS, MO 63102

EXAMINER
PARAS JR, PETER

ART UNIT	PAPER NUMBER
1632	

DATE MAILED: 10/18/2002 4

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/901,419	HALE ET AL.	
	Examiner	Art Unit	
	Peter Paras, Jr.	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____ .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-24* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-24* is/are rejected.

7)☒ Claim(s) *18* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *26 November 2001* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☒ Interview Summary (PTO-413) Paper No(s). *11* .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

The previous Office action mailed on 10/3/02 is hereby vacated in view of the instant Office action.

Applicant's preliminary amendments filed on 9/25/01 and 7/25/02 have been entered. Claims 1, 8, 11-12, 19, and 20 have been amended. New claim 24 has been added. Claims 1-24 are pending and are under current consideration.

Sequence Compliance

This application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37CFR 1.821(a)(1) and (a)(2). However, this application fails to comply with the requirements of 37 CFR 1.821 through 1.825 for the reason(s) set forth on the attached **N**otice To Comply With Requirements For Patent Applications Containing **N**ucleotide Sequence And/Or Amino Acid Sequence Disclosures. See the unidentified sequences on page 10 of the specification.

Applicants are required to comply with all of the requirements of 37 C.F.R. §§ 1.821 through 1.825. *Any* response to this Office Action, which fails to meet all of these requirements, will be considered non-responsive. The nature of the noncompliance with the requirements of 37 C.F.R. §§ 1.821 through 1.825 did not preclude the examination of the application on the merits, the results of which are communicated below.

Claim Objections

Claim 18 is objected to because of the following informalities: it appears that the term "connexin" is misspelled. Appropriate correction is required.

Claim Rejections - 35 USC § 112, 1st paragraph

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-4, 6-20 and 24 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for the claimed methods with respect to use of baculovirus vectors for infecting insect larvae, does not reasonably provide enablement for the claimed methods which embrace the use of other vectors that infect insect larvae. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The claims are directed to methods of producing a recombinant membrane protein in an insect larvae system comprising infecting larvae with a vector that has a nucleic acid sequence that encodes a recombinant membrane fusion protein with an affinity tag, particularly a poly His tag, wherein the recombinant membrane protein is expressed in the larvae and purifying the recombinant membrane protein by affinity chromatography. The claims are further directed to isolating the protein fraction from

the larvae by centrifugation. The claims also embrace different classes of membrane proteins such as NCX1, CFTR, and conexin 32.

The specification discusses that the invention features a method for producing a recombinant protein in an insect larvae expression system. See page 4, 3rd paragraph. The specification discusses that the invention features infection of insect larvae with a vector that has a nucleic acid sequence encoding a recombinant fusion protein of interest with an attached affinity tag. See pages 3-4. While the specification provides extensive teachings pertaining to the use of baculovirus vectors for infecting insect larvae the specification fails to provide any relevant teachings or specific guidance with regard to the use of the other vectors for infecting insect larvae embraced by the claims. Finally, the specification has exemplified the production of membrane fusion proteins, which is consistent with the proteins embraced by the claims, by providing working examples that teach the production of NCX1 and conexin 32. Given the lack of guidance provided by the specification it would have required undue experimentation to practice the claimed methods with the other vectors embraced by the claims.

While the specification has provided guidance for using a baculovirus vector to infect insect larvae the specification has not provided relevant teachings or guidance for use of other vectors to infect insect larvae embraced by the claims. The specification has contemplated that other vectors may be used to infect insect larvae. See the specification beginning on page 11 at the bottom and bridging to page 12. However, the specification has failed to recite which other vectors could be used to practice the claimed methods. Moreover, the specification has failed to provide any guidance,

working examples, or relevant teachings that would allow the skilled artisan to use vectors other than a baculovirus vector when practicing the claimed invention and the specification has not provided any correlation between use of a baculovirus and any other vector in the claimed methods so that the skilled artisan could extrapolate use of a baculovirus to use of other vectors. As previously stated the specification has not even identified which other vectors could be used to practice the claimed invention. A mere statement that other vectors exist and could be used is not sufficient to enable the breadth of the methods as directed to any vector that can infect insect larvae. If there is no disclosure of starting material or of any conditions under which claimed process can be carried out, undue experimentation is required, and there is failure to meet enablement requirement that cannot be rectified by asserting that all disclosure related to process is within skill of art. See Genentech Inc. v. Novo Nordisk A/S 42 USPQ2d 1001, 1997. In this case the starting material that has not been disclosed is any other vector that can infect insect larvae embraced by the claims.

Given the lack of guidance provided by the instant specification for use of other vectors to infect insect larvae it would have required undue experimentation for one skilled in the art to make and/or use the claimed invention.

Claims 1-4, 6-20 and 24 rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The claims are directed to methods of producing a recombinant membrane protein in an insect larvae system comprising infecting larvae with a vector that has a nucleic acid sequence that encodes a recombinant membrane fusion protein with an affinity tag, particularly a poly His tag, wherein the recombinant membrane protein is expressed in the larvae and purifying the recombinant membrane protein by affinity chromatography. The claims are further directed to isolating the protein fraction from the larvae by centrifugation. The claims also embrace different classes of membrane proteins such as NCX1, CFTR, and conexin 32.

Vas-Cath Inc. v. Mahurkar, 19USPQ2d 1111 (Fed. Cir. 1991), clearly states that "applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession *of the invention*. The invention is, for purposes of the 'written description' inquiry, *whatever is now claimed.*" Vas-Cath Inc. v. Mahurkar, 19USPQ2d at 1117. The specification does not "clearly allow persons of ordinary skill in the art to recognize that [he or she] invented what is claimed." Vas-Cath Inc. v. Mahurkar, 19USPQ2d at 1116.

While the specification has provided a description for a baculovirus vector that infects insect larvae for use in the claimed method, the specification has failed to provide a description for the other vectors that can infect insect larvae embraced by the claims. Based upon the prior art there is expected to be structure variation among the species of vectors that can infect insect larvae. The specification has not disclosed which other vectors could be used for infecting insect larvae to practice the claimed methods. There is no evidence on the record of a relationship between the structures of

baculovirus vectors and other vectors embraced by the claims that would provide any reliable information about the structure of vectors within the genus of vectors that infect insect cells. There is no evidence on the record that the vectors had known structural relationships to each other; the art indicated that there is variation between structures of vectors that infect insect cells. The claimed invention as a whole is not adequately described if the claims require essential or critical elements which are not adequately described in the specification and which is not conventional in the art as of applicants effective filing date. Possession may be shown by actual reduction to practice, clear depiction of the invention in a detailed drawing, or by describing the invention with sufficient relevant identifying characteristics such that a person skilled in the art would recognize that the inventor had possession of the claimed invention. Pfaff v. Wells Electronics, Inc., 48 USPQ2d 1641, 1646 (1998).

In the instant case the claimed embodiments of vectors that infect insect larvae, other than a baculovirus vector, encompassed within the genus of vectors that infect insect larvae lack a written description. The specification fails to describe what vectors fall into this genus. The skilled artisan cannot envision the detailed chemical structure of the encompassed vectors, and therefore conception is not achieved until reduction to practice has occurred, regardless of the complexity or simplicity of the method of isolation. Adequate written description requires more than a mere statement that it is part of the invention and reference to a potential method of isolating it. See *Fiers v. Revel*, 25 USPQ2d 1601, 1606 (Fed. Cir. 1993) and *Amgen Inc. v. Chugai Pharmaceutical Co. Ltd.*, 18 USPQ2d 1016 (Fed. Cir. 1991).

One cannot describe what one has not conceived. See *Fiddes v. Baird*, 30 USPQ2d 1481, 1483. In *Fiddes*, claims directed to mammalian FGF's were found to be unpatentable due to lack of written description for that broad class. The specification provided only the bovine sequence.

In view of the above considerations one of skill in the art would not recognize that applicant was in possession of the necessary common features or attributes possessed of the genus of vectors that infect insect larvae. Only a baculovirus vector has been described. Moreover, the art has recognized that there would be structural variation among the species of the genus of vectors that infect insect larvae. Therefore, Applicant was not in possession of the genus of vectors that infect insect larvae as encompassed by the claims. University of California v. Eli Lilly and Co., 43 USPQ2d 1398, 1404, 1405 held that to fulfill the written description requirement, a patent specification must describe an invention and do so in sufficient detail that one skilled in the art can clearly conclude that "the inventor invented the claimed invention."

Claim Rejections - 35 USC § 112, 2nd paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-24 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1 is confusing as written. The claim is confusing as written because the preamble is directed to producing a recombinant membrane fusion protein however, step (a) of the method recites that the nucleic acid sequence contained within the vector encoding a recombinant membrane fusion protein and then goes on to recite that the recombinant membrane protein is expressed and purified (step b). As such it is unclear which if recombinant membrane fusion protein or the recombinant membrane protein is intended to be produced and purified. Claims 2-24 depend from claim 1.

Claim 2 recites the limitation " the recombinant fusion protein " in line 1. There is insufficient antecedent basis for this limitation in the claim.

The term "substantially the same" in claim 19 is a relative term which renders the claim indefinite. The term "substantially the same" with respect to biological activity of the native form of the protein is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. The specification has not provided an adequate definition of what substantially the same biological activity means. The definition provided by the specification on page 6 merely states that the recombinant may be able to perform substantially the same function as the native form and but not define substantially the same biological activity as the native protein because it is unclear what substantially the same function means.

The term "substantially the same" in claim 20 is a relative term which renders the claim indefinite. The term "substantially the same" with respect to structure of the native form of the protein is not defined by the claim, the specification does not provide a

standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. The specification has not provided an adequate definition of what substantially the same structure means. The definition provided by the specification on page 6 merely states that the recombinant may have substantially the same tertiary and quaternary structure as the native form and but not define substantially the same structure as the native protein because it is unclear what substantially the same tertiary and quaternary structure means.

Claim 21 is incomplete as written. The claim is directed to a method of identifying the physical characteristics of a recombinant fusion protein. The claim however has not provided method steps identify the physical characteristics of a recombinant fusion protein so the goal of the preamble is set forth in a positive process. Claims 22-23 depend from claim 21 but appear to encompass method steps as the procedure used for identifying is disclosed.

Claim 21 is also indefinite as written because it encompasses a recombinant fusion protein produced by the method of claim 1. However, claim 1 is indefinite as set forth above because it is unclear which protein is being produced. Is it the recombinant membrane fusion protein or the recombinant membrane protein? As such it is unclear which protein is to be used in the method of claim 21 as the claim recites even broader language directed to a recombinant fusion protein. It is clear that recombinant fusion proteins, recombinant membrane fusion proteins, and recombinant fusion proteins can be different. As such the claim is confusing as written. Clarification is required. Claims 22-23 depend from claim 21.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 21-23 are rejected under 35 U.S.C. 102(b) as being anticipated by Pandit et al (US 5,866,114).

The claims are directed to methods for identifying the physical characteristics of a recombinant fusion protein produced by claim 1. The limitation introduced to the protein by claim 1 would be the affinity tag. It does appear that production of the protein in insect larvae would confer any unique characteristics to the protein.

Pandit et al teach that the physical characteristics of the M-CSF receptor (see the abstract) can be analyzed by crystallography. See columns 2-3. Pandit et al teach that the M-CSF receptor can comprise an affinity tag. See Example 5, column 13 bridging to column 14.

Thus, the teachings of Pandit meet all of the instant claim limitations.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-13, 19-21 and 24 are rejected under 35 U.S.C. 103(a) as being unpatentable over Au-Young (US 5,843,714) taken with Cha et al (Biotechnol. Prog., 1999, 15: 283-286) and Cha et al (Biotechnol. Bioeng., 1999, 65: 316-324; IDS #16).

The claims are directed to methods for producing a recombinant membrane protein in an insect larvae expression system, comprising infecting larvae with a vector comprising a nucleic acid sequence encoding a recombinant membrane fusion protein with an affinity tag, wherein the protein is expressed in larvae and purified by affinity chromatography, and wherein the affinity tag is poly (His), and wherein the vector is a baculovirus, wherein the recombinant membrane protein can be a transport protein. The claims are further directed to infecting the larvae at the fourth instar stage of development, centrifuging an extract from larvae to isolate a fraction comprising the recombinant membrane fusion protein and infecting the insect larvae by injecting the vector.

Au-Young teach that PLHu is a membrane protein that is a member of the class of small hydrophobic transport-related proteolipids containing four alpha-helical transmembrane domains. See column 4, lines 43-45. Au-Young teach that biologically active PLHu can be produced by inserting the nucleotide sequence encoding PLHu into an expression vector of choice for use in an appropriate host system. See column 8, lines 28-54. Au-Young contemplate that a method of producing human proteolipid

(PLHu) would comprise an insect larvae expression system, comprising infecting Trichoplusia larvae with an Autographa californica nuclear polyhedrosis virus (AcNPV, which is a baculovirus) vector comprising the coding sequence of PLHu (see column 9, beginning in line 56 and bridging to column 10 through line 2), wherein PLHu can be expressed as a fusion protein comprising additional polypeptide domains added to facilitate protein purification, in particular histidine-tryptophan, protein A domains, and FLAG domains can be added to facilitate affinity purification; the histidine residues allow for purification by affinity chromatography and can be cleaved from the proteolipid by an enterokinase. See column 12 lines 34-54. Au-Young teach that an alternative method to purify PLHu entails use of an immunoaffinity column that comprises a PLHu antibody that would bind and capture PLHu as it passes through the column. Au-Young teach that cellular fractions from cells containing PLHu are prepared by solubilization of whole cells and isolation of subcellular fractions by differential centrifugation; the PLHu-containing fractions are then passed over the immunoaffinity column and PLHu is subsequently eluted and collected. See column 28, lines 19-27. Au-Young contemplate that PLHu may form a pore in the lipid bilayer of cellular membranes based on its structural homology to other proteolipids and propose a method to assay the pore-forming ability of purified recombinant PLHu, which is interpreted to read on a method of determining the physical characteristics of PLHu. See column 27, lines 15-27.

Au-Young do not teach actual production and purification of a PLHu fusion protein from insect larvae.

However, at the time the claimed invention was made, methods of producing a recombinant protein in insect larvae were within the routine skill of the ordinary artisan as evidenced by Cha et al (A, Biotechnol. Prog.) and Cha et al (B, Biotechnol. Bioeng). It is noted that although Cha (A) and Cha (B) do not teach production of recombinant membrane fusion proteins in insect larvae, absent evidence to the contrary, one of ordinary skill in the art would have a reasonable expectation that the methodology of Cha (A) and Cha (B) could be extrapolated to encompass production of recombinant membrane fusion proteins. In particular, Cha et al (A) teach a production of fusion proteins comprising a histidine affinity ligand [for simplified purification using affinity chromatography], gfp, and enterokinase cleavage site [for recovery of the product from the fusion], and the product [protein of interest, demonstrating by way of example Cha et al taught purification of human interleukin-2 (hIL-2)]. See Figure 1 on page 283. Cha et al teach that a nucleotide sequence encoding such a fusion protein is inserted into a baculovirus vector, which was then used to infect insect larvae at the fourth instar stage of development. See the Materials and Methods section: Strains and Recombinant Baculoviruses beginning on page 283 and bridging to page 284. Cha et al go on to report that the larvae are homogenized in a buffer to produce a homogenate containing the fusion protein. The homogenate is then centrifuged to remove debris and the supernatant comprising the fusion protein is used for purification. See the Materials and Methods section: Sample Preparation and Storage on page 284. The fusion protein was then affinity purified on an affinity column by immobilized metal affinity chromatography (IMAC). See page 284 column 2. Purified fusion protein is then eluted

from the IMAC column. The fractions containing the fusion protein can be visualized by examining the fluorescence of the GFP under UV light. See figure 3 on page 285. The eluted purified samples were then assayed by SDS-PAGE and Western blot. The purified fusion protein was then cleaved by an enterokinase to separate hIL-2 from the GFP/His. See Figure 5 on page 285 as well as column 2 on page 285. Cha et al discuss that the hIL-2 was cloned in frame (see page 285, the first line of the Conclusion section), which can be interpreted to mean that recombinantly produced hIL-2 would have substantially the same structure and activity as the native form of hIL-2; Cha et al also report that recombinantly produced GFP produced by the same method fluoresces under UV light, which is interpreted by mean that it has the substantially the same structure and activity as the native form and is also broadly interpreted to be a method for identifying the physical characteristics of recombinant GFP. Also see Cha et al (B) who report that infecting the insect larvae may also be accomplished by injecting a recombinant virus into the cuticle of insect larvae. See page 317, column 1 at the beginning of the first full paragraph.

Accordingly, in view of the routine state of the art of protein production in insect larvae as presented by Cha (A) and Cha (B), it would have been obvious to produce a recombinant PLHu fusion protein in an insect larvae system. One of ordinary skill in the art would have been sufficiently motivated to produce a PLHu fusion protein in an insect larvae system because it was an art-recognized goal to produce recombinant PLHu as taught by Au-Young (see column 8, lines 28-54), and in particular to use an insect larvae as discussed by Au-Young (see column 9, beginning in line 56 and bridging to

column 10 through line 2), and more particularly because the methods of protein production in insect larvae as exemplified by Cha (A) and Cha (B) have a general utility for rapid and identification and purification of recombinant proteins expressed by baculovirus (see Cha (A) on page 286).

Thus, the claimed invention, as a whole, was clearly prima facie obvious in the absence of evidence to the contrary.

Claims 1-12, 15, 19-21 and 24 are rejected under 35 U.S.C. 103(a) as being unpatentable over Goli et al (US 5,854,411) taken with Cha et al (Biotechnol. Prog., 1999, 15: 283-286) and Cha et al (Biotechnol. Bioeng., 1999, 65: 316-324; IDS#16).

The claims are directed to methods for producing a recombinant membrane protein in an insect larvae expression system, comprising infecting larvae with a vector comprising a nucleic acid sequence encoding a recombinant membrane fusion protein with an affinity tag, wherein the protein is expressed in larvae and purified by affinity chromatography, and wherein the affinity tag is poly (His), and wherein the vector is a baculovirus, wherein the recombinant membrane protein can be a channel forming protein. The claims are further directed to infecting the larvae at the fourth instar stage of development, centrifuging an extract from larvae to isolate a fraction comprising the recombinant membrane fusion protein and infecting the insect larvae by injecting the vector.

Goli et al teach that HCCP is a human chloride channel. See column 7 lines 47-51. Goli et al teach that biologically active HCCP can be produced by inserting the nucleotide sequence encoding HCCP into an expression vector of choice for use in an appropriate host system. See column 11, beginning on line 55 and bridging to column 12, through line 15. Goli et al contemplate that a method of producing HCCP would comprise an insect larvae expression system, comprising infecting Trichoplusia larvae with an Autographa californica nuclear polyhedrosis virus (AcNPV, which is a baculovirus) vector comprising the coding sequence of HCCP (see column 13, lines 16-28), wherein HCCP can be expressed as a fusion protein comprising additional polypeptide domains added to facilitate protein purification, in particular histidine-tryptophan, protein A domains, and FLAG domains can be added to facilitate affinity purification; the histidine residues allow for purification by affinity chromatography and can be cleaved from HCCP by an enterokinase. See column 15 beginning on line 45 and bridging to column 16, line 10. Goli et al teach that an alternative method to purify HCCP entails use of an immunoaffinity column that comprises a HCCP antibody that would bind and capture HCCP as it passes through the column. Goli et al teach that medium, containing HCCP, is passed over the immunoaffinity column and HCCP is subsequently eluted and collected. See column 32, lines 4-11. Goli et al assert that HCCP is a chloride channel and propose a method to assay the chloride conductance ability of recombinant HCCP by transforming cells *in vitro* with an HCCP expression vector and measuring chloride conductance before and after incubation of an HCCP specific antibody that will block the pore in the ion channel and prevent chloride from

passing, which is interpreted to read on a method of determining the physical characteristics of HCCP. See column 30 beginning in line 60 and bridging to column 31, through line 21.

Goli et al do not teach actual production and purification of a HCCP fusion protein from insect larvae.

However, at the time the claimed invention was made, methods of producing a recombinant protein in insect larvae were within the routine skill of the ordinary artisan as evidenced by Cha et al (A, Biotechnol. Prog.) and Cha et al (B, Biotechnol. Bioeng). It is noted that although Cha (A) and Cha (B) do not teach production of recombinant membrane fusion proteins in insect larvae, absent evidence to the contrary, one of ordinary skill in the art would have a reasonable expectation that the methodology of Cha (A) and Cha (B) could be extrapolated to encompass production of recombinant membrane fusion proteins. In particular, Cha et al (A) teach a production of fusion proteins comprising a histidine affinity ligand [for simplified purification using affinity chromatography], gfp, and enterokinase cleavage site [for recovery of the product from the fusion], and the product [protein of interest, demonstrating by way of example Cha et al taught purification of human interleukin-2 (hIL-2)]. See Figure 1 on page 283. Cha et al teach that a nucleotide sequence encoding such a fusion protein is inserted into a baculovirus vector, which was then used to infect insect larvae at the fourth instar stage of development. See the Materials and Methods section: Strains and Recombinant Baculoviruses beginning on page 283 and bridging to page 284. Cha et al go on to report that the larvae are homogenized in a buffer to produce a homogenate containing

the fusion protein. The homogenate is then centrifuged to remove debris and the supernatant comprising the fusion protein is used for purification. See the Materials and Methods section: Sample Preparation and Storage on page 284. The fusion protein was then affinity purified on an affinity column by immobilized metal affinity chromatography (IMAC). See page 284 column 2. Purified fusion protein is then eluted from the IMAC column. The fractions containing the fusion protein can be visualized by examining the fluorescence of the GFP under UV light. See figure 3 on page 285. The eluted purified samples were then assayed by SDS-PAGE and Western blot. The purified fusion protein was then cleaved by an enterokinase to separate hIL-2 from the GFP/His. See Figure 5 on page 285 as well as column 2 on page 285. Cha et al discuss that the hIL-2 was cloned in frame (see page 285, the first line of the Conclusion section), which can be interpreted to mean that recombinantly produced hIL-2 would have substantially the same structure and activity as the native form of hIL-2; Cha et al also report that recombinantly produced GFP produced by the same method fluoresces under UV light, which is interpreted by mean that it has the substantially the same structure and activity as the native form and is also broadly interpreted to be a method for identifying the physical characteristics of recombinant GFP. Also see Cha et al (B) who report that infecting the insect larvae may also be accomplished by injecting a recombinant virus into the cuticle of insect larvae. See page 317, column 1 at the beginning of the first full paragraph.

Accordingly, in view of the routine state of the art of protein production in insect larvae as presented by Cha (A) and Cha (B), it would have been obvious to produce a

recombinant HCCP fusion protein in an insect larvae system. One of ordinary skill in the art would have been sufficiently motivated to produce an HCCP protein fusion in an insect larvae system because it was an art-recognized goal to produce recombinant HCCP as taught by Goli et al (see column 11, beginning on line 55 and bridging to column 12, through line 15), and in particular to use an insect larvae as discussed by Goli et al (see column 13, lines 16-28), and more particularly because the methods of protein production in insect larvae as exemplified by Cha (A) and Cha (B) have a general utility for rapid and identification and purification of recombinant proteins expressed by baculovirus (see Cha (A) on page 286).

Thus, the claimed invention, as a whole, was clearly prima facie obvious in the absence of evidence to the contrary.

Claims 1-12, 19-21, and 24 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hillman et al (US 6,033,870) taken with Cha et al (Biotechnol. Prog., 1999, 15: 283-286) and Cha et al (Biotechnol. Bioeng., 1999, 65: 316-324; IDS#16).

The claims are directed to methods for producing a recombinant membrane protein in an insect larvae expression system, comprising infecting larvae with a vector comprising a nucleic acid sequence encoding a recombinant membrane fusion protein with an affinity tag, wherein the protein is expressed in larvae and purified by affinity chromatography, and wherein the affinity tag is poly (His), and wherein the vector is a baculovirus. The claims are further directed to infecting the larvae at the fourth instar stage of development, centrifuging an extract from larvae to isolate a fraction

comprising the recombinant membrane fusion protein and infecting the insect larvae by injecting the vector.

Hillman et al teach that TMP-2 is a human integral membrane protein. Hillman et al teach that biologically active TMP-2 can be produced by inserting the nucleotide sequence encoding TMP-2 into an expression vector of choice for use in an appropriate host system. See column 15, in lines 24-52. Hillman et al contemplate that a method of producing TMP-2 would comprise an insect larvae expression system, comprising infecting Trichoplusia larvae with an Autographa californica nuclear polyhedrosis virus (AcNPV, which is a baculovirus) vector comprising the coding sequence of TMP-2 (see column 16, lines 54-67), wherein TMP-2 can be expressed as a fusion protein comprising additional polypeptide domains added to facilitate protein purification, in particular histidine-tryptophan, protein A domains, and FLAG domains can be added to facilitate affinity purification; the histidine residues allow for purification by affinity chromatography and can be cleaved from TMP-2 by an enterokinase. See column 19 lines 41-64. Hillman et al teach that an alternative method to purify TMP-2 entails use of an immunoaffinity column that comprises a TMP-2 antibody that would bind and capture TMP-2 as it passes through the column. Hillman et al teach that medium, containing TMP-2, is passed over the immunoaffinity column and TMP-2 is subsequently eluted and collected. See column 36, lines 10-26. Hillman provides an assay for determining the activity of TMP-2 by assessing its effect on cell motility, for example, wherein an increase in motility of cultured cells indicates that recombinant TMP-2 has substantially the same biological activity and structure as native TMP-2 as it

is suggested that native TMP-2 is related to metastatic potential of breast and kidney carcinomas, which is interpreted to read on a method of determining the physical characteristics of TMP-2. See Example IX beginning in column 34 and bridging to column 35.

Hillman et al do not teach actual production and purification of a TMP-2 fusion protein from insect larvae.

However, at the time the claimed invention was made, methods of producing a recombinant protein in insect larvae were within the routine skill of the ordinary artisan as evidenced by Cha et al (A, Biotechnol. Prog.) and Cha et al (B, Biotechnol. Bioeng). It is noted that although Cha (A) and Cha (B) do not teach production of recombinant membrane fusion proteins in insect larvae, absent evidence to the contrary, one of ordinary skill in the art would have a reasonable expectation that the methodology of Cha (A) and Cha (B) could be extrapolated to encompass production of recombinant membrane fusion proteins. In particular, Cha et al (A) teach a production of fusion proteins comprising a histidine affinity ligand [for simplified purification using affinity chromatography], gfp, and enterokinase cleavage site [for recovery of the product from the fusion], and the product [protein of interest, demonstrating by way of example Cha et al taught purification of human interleukin-2 (hIL-2)]. See Figure 1 on page 283. Cha et al teach that a nucleotide sequence encoding such a fusion protein is inserted into a baculovirus vector, which was then used to infect insect larvae at the fourth instar stage of development. See the Materials and Methods section: Strains and Recombinant Baculoviruses beginning on page 283 and bridging to page 284. Cha et al go on to

report that the larvae are homogenized in a buffer to produce a homogenate containing the fusion protein. The homogenate is then centrifuged to remove debris and the supernatant comprising the fusion protein is used for purification. See the Materials and Methods section: Sample Preparation and Storage on page 284. The fusion protein was then affinity purified on an affinity column by immobilized metal affinity chromatography (IMAC). See page 284, column 2. Purified fusion protein is then eluted from the IMAC column. The fractions containing the fusion protein can be visualized by examining the fluorescence of the GFP under UV light. See figure 3 on page 285. The eluted purified samples were then assayed by SDS-PAGE and Western blot. The purified fusion protein was then cleaved by an enterokinase to separate hIL-2 from the GFP/His. See Figure 5 on page 285 as well as column 2 on page 285. Cha et al discuss that the hIL-2 was cloned in frame (see page 285, the first line of the Conclusion section), which can be interpreted to mean that recombinantly produced hIL-2 would have substantially the same structure and activity as the native form of hIL-2; Cha et al also report that recombinantly produced GFP produced by the same method fluoresces under UV light, which is interpreted by mean that it has the substantially the same structure and activity as the native form and is also broadly interpreted to be a method for identifying the physical characteristics of recombinant GFP. Also see Cha et al (B) who report that infecting the insect larvae may also be accomplished by injecting a recombinant virus into the cuticle of insect larvae. See page 317, column 1 at the beginning of the first full paragraph.

Accordingly, in view of the routine state of the art of protein production in insect larvae as presented by Cha (A) and Cha (B), it would have been obvious to produce a recombinant TMP-2 fusion protein in an insect larvae system. One of ordinary skill in the art would have been sufficiently motivated to produce a TMP-2 fusion protein in an insect larvae system because it was an art-recognized goal to produce recombinant TMP-2 as taught by Hillman et al (see column 15, in lines 24-52), and in particular to use an insect larvae as discussed by Hillman et al (see column 16, lines 54-67), and more particularly because the methods of protein production in insect larvae as exemplified by Cha (A) and Cha (B) have a general utility for rapid and identification and purification of recombinant proteins expressed by baculovirus (see Cha (A) on page 286).

Thus, the claimed invention, as a whole, was clearly prima facie obvious in the absence of evidence to the contrary.

Conclusion

No claim is allowed. Claims 14 and 16-18 appear to be free of the prior art of record but are subject to other rejections.

Any inquiry concerning this communication or earlier communications from the examiner(s) should be directed to Peter Paras, Jr., whose telephone number is 703-308-8340. The examiner can normally be reached Monday-Friday from 8:30 to 4:30 (Eastern time).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Deborah Reynolds, can be reached at 703-305-4051. Papers related to this application may be submitted by facsimile transmission. Papers should be faxed via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). The CM1 Fax Center numbers are (703) 308-4242 and (703) 305-3014.

Inquiries of a general nature or relating to the status of the application should be directed to Patsy Zimmerman whose telephone number is (703) 308-0009.

Peter Paras, Jr.

Art Unit 1632

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Art Unit 1632